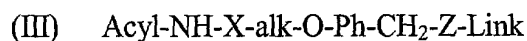


## WHAT IS CLAIMED IS:

1. A compound of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , a carbonyl of formula  $-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B-\text{C}(\text{O})-\text{NR}-$ , an amide bond of formula  $-(\text{CH}_2)_B-\text{NR}-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 5 carbon atoms;

Ph is a phenyl group optionally substituted with one or more methoxy or nitro groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

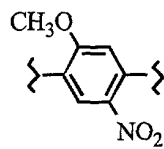
Epitope Tag Site is a sequence of amino acids,

where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease.

2. The compound of Claim 1, wherein said compound is selected from the group consisting of Acyl-NH-CASENLYFQ GK-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-NH-C(O)-CH<sub>2</sub>I, Acyl-NH-CASENLYFQGOrn-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-NH-C(O)-CH<sub>2</sub>I, Acyl-NH-CASENLYFQGPK-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-NH-C(O)-CH<sub>2</sub>I, and Acyl-NH-CASENLYFQGPOrn-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-NH-C(O)-CH<sub>2</sub>I.

3. The compound of Claim 1, wherein said alk is a straight chain alkylene selected from the group consisting of methylene, ethylene, propylene, n-butylene, and n-pentylene.



4. The compound of Claim 1, wherein said Ph is

5. The compound of Claim 1, wherein said Z is a single amino acid.

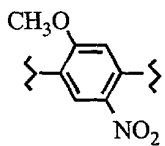
6. The compound of Claim 5, wherein said Z is selected from the group consisting of glycine, alanine, and valine.

7. The compound of Claim 1, wherein said Z is a synthetic amino acid.

8. The compound of Claim 7, wherein said synthetic amino acid contains an amino group in a position selected from the group consisting of  $\beta$ ,  $\delta$ ,  $\epsilon$ ,  $\phi$ , or  $\gamma$  to the carboxyl group.

9. The compound of Claim 7, wherein said Z is  $\gamma$ -aminobutyric acid.

10. The compound of Claim 1, wherein said compound is selected from the group consisting of: Acyl-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-O-Ph-CH<sub>2</sub>-G-NH-C(O)-CH<sub>2</sub>I, Acyl-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-O-Ph-CH<sub>2</sub>-A-NH-C(O)-CH<sub>2</sub>I, Acyl-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-O-Ph-CH<sub>2</sub>- $\gamma$ -aminobutyric acid-NH-C(O)-CH<sub>2</sub>I, and Acyl-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-O-Ph-CH<sub>2</sub>-V-NH-C(O)-CH<sub>2</sub>I,

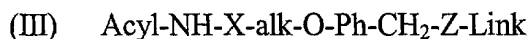


where Ph is

11. A method for simultaneously identifying and determining the levels of expression of cysteine-containing proteins in normal and perturbed cells, comprising:

a) preparing a first protein sample or a first peptide sample from the normal cells;

b) reacting the first protein sample or the first peptide sample with a reagent of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C(O)}-\text{NR}-$ , a carbonyl of formula  $-\text{C(O)}-$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C(O)}-\text{NR}-$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B-\text{C(O)}-\text{NR}-$ , an amide bond of formula  $-(\text{CH}_2)_B-\text{NR}-\text{C(O)}-$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 10 carbon atoms;

Ph is a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

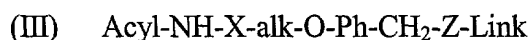
Epitope Tag Site is a sequence of amino acids,

where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease;

c) preparing a second protein sample or a second peptide sample from the perturbed cells;

d) reacting the second protein sample or the second peptide sample of step c) with a second reagent of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , a carbonyl of formula  $-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B-\text{C}(\text{O})-\text{NR}-$ , an amide bond of formula  $-(\text{CH}_2)_B-\text{NR}-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 10 carbon atoms;

Ph is a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

Epitope Tag Site is a sequence of amino acids,

where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease,

such that the molecular weight of the first reagent and the molecular weight of the second reagent are different by an integer multiple of 14 atomic mass units;

e) combining the reacted the first and the second protein samples or the reacted the first and the second peptide sample from steps b) and d);

f) subjecting the combined protein samples or the combined peptide samples from step e) to proteolysis at a site on the protein samples or at a site on the peptide samples, the site being other than the Protease Cleavage Site;

g) subjecting the proteolyzed combined protein samples or the proteolyzed peptide samples from step f) to an affinity chromatography system comprising a second amino acid sequence attached to a solid, thereby forming bound proteins and non-bound proteins,

where the Epitope Tag Site of the reagent and the second amino acid sequence bind with high specificity to each other;

h) eluting the non-bound proteins from the affinity chromatography system;

i) subjecting the affinity chromatography system from step h) to a TEV protease, thereby forming a cleaved protein mixture;

j) eluting the cleaved protein mixture from the affinity chromatography system of step i);

k) isolating the eluted protein mixture obtained from step j);

l) subjecting the eluted protein mixture from step k) to chromatographic separation, followed by mass analysis;

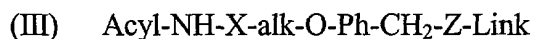
m) comparing the results of step l) to:

1) determining the ratio of amounts of compounds in the two samples, where the molecular weights thereof are separated by an integer multiple of 14 atomic mass units; and

2) comparing the results obtained for each compound to protein databases containing chromatographic and molecular weight correlations.

12. A method for simultaneously identifying and determining the levels of expression of cysteine-containing proteins in normal and perturbed cells, comprising:

- a) preparing a first protein sample or a first peptide sample from the normal cells;
- b) reacting the first protein sample or the first peptide sample with a reagent of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , a carbonyl of formula  $-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B-\text{C}(\text{O})-\text{NR}-$ , an amide bond of formula  $-(\text{CH}_2)_B-\text{NR}-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 10 carbon atoms;

Ph is a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

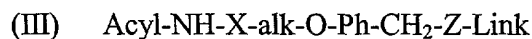
Epitope Tag Site is a sequence of amino acids,

where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease;

c) preparing a second protein sample or a second peptide sample from the perturbed cells;

d) reacting the second protein sample or the second peptide sample of step c) with a second reagent of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , a carbonyl of formula  $-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B-\text{C}(\text{O})-\text{NR}-$ , an amide bond of formula  $-(\text{CH}_2)_B-\text{NR}-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 10 carbon atoms;

Ph is a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

Epitope Tag Site is a sequence of amino acids,

where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease,

such that the molecular weight of the first reagent and the molecular weight of the second reagent are different by an integer multiple of 14 atomic mass units;

e) combining the reacted the first and the second protein samples or the reacted the first and the second peptide sample from steps b) and d);

f) subjecting the combined protein samples or the combined peptide samples from step e) to proteolysis at a site on the protein samples or at a site on the peptide samples, the site being other than the Protease Cleavage Site;

g) subjecting the proteolyzed combined protein samples or the proteolyzed peptide samples from step f) to an affinity chromatography system comprising a second amino acid sequence attached to a solid, thereby forming bound proteins and non-bound proteins,

where the Epitope Tag Site of the reagent and the second amino acid sequence bind with high specificity to each other;

h) eluting the non-bound proteins from the affinity chromatography system;

i) subjecting the affinity chromatography system from step h) to TEV protease, thereby forming a cleaved protein mixture;

j) eluting the cleaved protein mixture from the affinity chromatography system of step i);

k) isolating the eluted protein mixture obtained from step j);

l) subjecting the eluted protein mixture from step k) to chromatographic separation, followed by mass analysis;

m) comparing the results of step l) to:

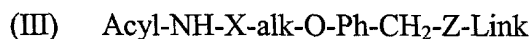
1) determining the ratio of amounts of compounds in the two samples, where the molecular weights thereof are separated by an integer multiple of 14 atomic mass units; and

2) comparing the results obtained for each compound to protein databases containing chromatographic and molecular weight correlations.



13. A method for simultaneously identifying and determining the levels of expression of cysteine-containing proteins in normal and perturbed cells, comprising:

- a) preparing a first protein sample or a first peptide sample from the normal cells;
- b) subjecting the first protein sample or the first peptide sample from step a) to proteolysis;
- c) reacting the proteolyzed first protein sample or the proteolyzed first peptide sample with a reagent of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C(O)-NR-}$ , a carbonyl of formula  $-\text{C(O)-}$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C(O)-NR-}$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B\text{-C(O)-NR-}$ , an amide bond of formula  $-(\text{CH}_2)_B\text{-NR-C(O)-}$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 10 carbon atoms;

Ph is a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

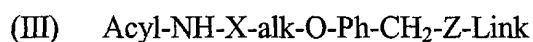
Epitope Tag Site is a sequence of amino acids,  
 where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease;

d) preparing a second protein sample or a second peptide sample from the perturbed cells;

e) subjecting the second protein sample or the second peptide sample from step d) to proteolysis;

f) reacting the proteolyzed second protein sample or the proteolyzed second peptide sample of step e) with a second reagent of Formula II or III:



where:

A is an integer from 0 to 12;

X is selected from the group consisting of an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , a carbonyl of formula  $-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 10 amino acids, where R is hydrogen or lower alkyl;

Y is an amide bond of formula  $-\text{C}(\text{O})-\text{NR}-$ , where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is selected from the group consisting of an amide bond of formula  $-(\text{CH}_2)_B-\text{C}(\text{O})-\text{NR}-$ , an amide bond of formula  $-(\text{CH}_2)_B-\text{NR}-\text{C}(\text{O})-$ , and an amino acid sequence comprising between 0 to 3 amino acids,

where R is hydrogen or lower alkyl, and

where B is an integer from 0 to 20;

alk is straight or branched chain of alkylene comprising between 0 and 10 carbon atoms;

Ph is a phenyl group optionally substituted with one or more electron withdrawing groups ortho or para to the  $-\text{CH}_2-$  group;

Link is selected from the group consisting of Lys- $\epsilon$ -iodoacetamide, Arg- $\delta$ -iodoacetamide, and Orn- $\delta$ -iodoacetamide;

Epitope Tag Site is a sequence of amino acids,

where when A is two or more, the amino acid sequence of each Epitope Tag Site can be the same or different; and

Protease Cleavage Site is an amino acid sequence of SEQ ID NO: 1 that is a cleavage site for TEV protease,

such that the molecular weight of the first reagent and the molecular weight of the second reagent are different by an integer multiple of 14 atomic mass units;

g) combining the reacted first and second protein samples or the reacted first and second peptide sample from steps c) and f);

h) subjecting the combined protein samples or the combined peptide samples from step e) to proteolysis at a site on the protein samples or at a site on the peptide samples, the site being other than the Protease Cleavage Site;

i) subjecting the proteolyzed combined protein samples or the proteolyzed peptide samples from step f) to an affinity chromatography system comprising a second amino acid sequence attached to a solid, thereby forming bound proteins and non-bound proteins,

where the Epitope Tag Site of the reagent and the second amino acid sequence bind with high specificity to each other;

j) eluting the non-bound proteins from the affinity chromatography system;

k) subjecting the affinity chromatography system from step j) to TEV protease, thereby forming a cleaved protein mixture;

l) eluting the cleaved protein mixture from the affinity chromatography system of step k);

m) isolating the eluted protein mixture obtained from step l);

n) subjecting the eluted protein mixture from step m) to a two-dimensional liquid chromatographic separation, wherein said dimensions are selected from the group consisting of size differentiation, charge differentiation, hydrophobicity, hydrophilicity, and polarity, followed by a two-dimensional mass analysis;

- o) comparing the results of step n) to:
  - 1) determining the ratio of amounts of compounds in the two samples, where the molecular weights thereof are separated by an integer multiple of 14 atomic mass units; and
  - 2) comparing the results obtained for each compound to protein databases containing chromatographic and molecular weight correlations;

wherein said Z substituent in the first reagent has a molecular weight that is an integer multiple of 14 atomic mass units different than the Z substituent in the second reagent.

14. The method of Claim 13, wherein said Link in step c) is Lys- $\epsilon$ -iodoacetamide, and said Link in step f) is Orn- $\delta$ -iodoacetamide.

15. The method of Claim 13, wherein said Link in step c) is Orn- $\delta$ -iodoacetamide, and said Link in step f) is Lys- $\epsilon$ -iodoacetamide.

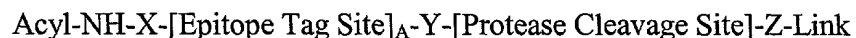
16. The method of Claim 13, wherein said reagent of step c) or step f) reacts with the reactive side chain of one or more amino acid residues of a protein in the first or second protein sample;

wherein said amino acid residue is selected from the group consisting of tyrosine, cysteine, proline, and histidine.

17. The method of Claim 16 wherein said amino acid residue is a cysteine.

18. A method for proteomic analysis, comprising:

- a) preparing a protein sample or a peptide sample from cells;
- b) reacting the protein sample or the peptide sample with a reagent of the formula:



where:

A is an integer from 1 to 12;

X is an amide bond of formula -C(O)-NR-, where R is hydrogen or lower alkyl, or X is an amino acid sequence comprising between 0 to 10 amino acids;

Y is an amide bond of formula -C(O)-NR-, where R is hydrogen or lower alkyl, or Y is an amino acid sequence comprising between 0 to 10 amino acids;

Z is an amide bond of formula -C(O)-NR-, where R is hydrogen or lower alkyl, or Z is an amino acid sequence comprising between 0 to 10 amino acids;

Link is selected from the group consisting of Lys-ε-iodoacetamide, Arg-δ-iodoacetamide, and Orn-δ-iodoacetamide;

Epitope Tag Site is a sequence of amino acids, and

Protease Cleavage Site is a sequence of amino acids that is a cleavage site for a highly specific protease enzyme;

c) subjecting the reacted proteins or peptides from step b) to proteolysis at a site on the protein samples or at a site on the peptide samples, the site being other than the Protease Cleavage Site;

d) subjecting the proteolyzed reacted proteins or the proteolyzed reacted peptides from step c) to an affinity chromatography system comprising a second amino acid sequence attached to a solid support, thereby forming bound proteins and non-bound proteins,

where the Epitope Tag Site of the reagent and the second amino acid sequence bind with high specificity to each other;

e) eluting the non-bound proteins from the affinity chromatography system;

f) subjecting the affinity chromatography system from step e) to TEV protease specific for the Protease Cleavage Site, thereby forming a cleaved protein mixture;

g) eluting the cleaved protein mixture from the affinity chromatography system of step f);

h) isolating the cleaved protein mixture obtained from step g);

i) subjecting the cleaved protein mixture from step h) to a two-dimensional chromatographic separation, wherein said dimensions of the multi-dimensional liquid chromatographic separation are selected from the group consisting of size differentiation, charge differentiation, hydrophobicity, hydrophilicity, and polarity, followed by a two-dimensional mass analysis;

j) comparing the results of step i) to:

1) determine the ratio of amounts of compounds in the sample separated by a molecular weight of 14 atomic mass units; and

2) identify the various modified proteins by comparing the results obtained for each modified protein to protein databases containing chromatographic and molecular weight correlations.

19. The method of Claim 18, wherein said reagent reacts with the reactive side chain of one or more of the amino acid residues of the first or second protein;

wherein said amino acid residue is selected from the group consisting of tyrosine, cysteine, proline, and histidine.

20. The method of Claim 19, wherein said amino acid residue is a cysteine.